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Amidolytic and Immuno-Nephelometric Determination of α_1-Proteinase Inhibitor and α_2-Macroglobulin in Serum with Calculation of Specific Inhibitor Activities in Health and Disease

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Summary: In sera of healthy persons (n = 50) and patients with a variety of diseases (n = 197) the two major proteinase inhibitors, α_1-proteinase inhibitor (α_1-antitrypsin) and α_2-macroglobulin, were measured by two methods: a chromogenic (amidolytic) substrate assay to assess the functional activities, and a laser nephelometric method to determine the immunoreactive concentrations of the respective proteins. The specific proteinase inhibitor activities defined as the number of inhibitor units per g inhibitor protein were calculated.

The precision and accuracy of both assays were found to be similar, showing a satisfactory correlation of results for the sera of healthy persons (r = 0.916 for α_2-macroglobulin and 0.988 for α_1-proteinase inhibitor). In diseased individuals the correlation was lower than in normal persons (0.862 for α_2-macroglobulin and 0.907 for α_1-proteinase inhibitor). A poor correlation was obtained in patients with liver diseases (r = 0.586 for α_1-proteinase inhibitor and 0.852 for α_2-macroglobulin).

Reference ranges were established for functional and immunological concentrations and for specific inhibitor activities, respectively. Normal values followed a *Gaussian* distribution.

In patients with various diseases including those with acute phase response, the specific inhibitor activities of α_1-proteinase inhibitor are reduced significantly; this is because inhibitor activity shows a smaller relative increase than immunoreactivity. Among the various diseases, no significant differences were noted. The specific inhibitor activity of α_2-macroglobulin changed significantly only in patients with carcinoma, liver diseases and trauma. Follow up of some patients shows also intraindividual variation of specific proteinase inhibitor activities.

Amidolytische und immun-nephelometrische Bestimmung von α_1-Proteinase-Inhibitor und α_2-Makroglobulin im Serum mit Berechnung der spezifischen inhibitorischen Aktivitäten bei Gesunden und Kranken

Zusammenfassung: In Sera von gesunden Personen (n = 50) und Patienten mit verschiedenen Erkrankungen (n = 197) wurden die beiden wesentlichen Proteinase-Inhibitoren α_1-Proteinase-Inhibitor (α_1-Antitrypsin) und α_2-Makroglobulin mit einem chromogenen (amidolytischen) Substrat-Test zur Ermittlung der funktionellen Aktivität und mit einer lasernephelometrischen Methode zur Quantifizierung der immunreaktiven Konzentration bestimmt. Die spezifischen Proteinase-Inhibitor-Aktivitäten, definiert als die Zahl der Inhibitoreinheiten pro Gramm Inhibitorprotein, wurden berechnet.

Präzision und Richtigkeit beider Bestimmungsprinzipien sind vergleichbar. Die Korrelation der Ergebnisse mit Seren von gesunden Personen ist zufriedenstellend (r = 0,916 für α_2-Makroglobulin und r = 0,988 für α_1-Proteinase-Inhibitor). Bei Erkrankungen ist diese Korrelation geringer als bei Gesunden (r = 0,862 für α_2-

Makroglobulin und r = 0,907 für α_1-Proteinase-Inhibitor). Eine geringe Korrelation wurde vor allem bei Patienten mit Lebererkrankungen (r = 0,586 für α_1-Proteinase-Inhibitor und r = 0,852 für α_2-Makroglobulin festgestellt.

Referenzbereiche wurden erstellt für funktionelle und immunreaktive Konzentrationen sowie für spezifische Inhibitoraktivitäten. Die Normalwerte folgen einer *Gauss*'schen Verteilung.

Bei Patienten mit verschiedenen Erkrankungen einschließlich solcher mit Akutphase-Reaktion ist die spezifische Inhibitoraktivität des α_1-Proteinase-Inhibitors signifikant vermindert, was auf einen relativ geringeren Anstieg der Inhibitoraktivität im Vergleich zur Immunreaktivität zurückzuführen ist. Zwischen den verschiedenen Erkrankungen ergeben sich keine Unterschiede.

Die spezifische Inhibitoraktivität des α_2-Makroglobulins verändert sich signifikant nur bei Patienten mit Carcinomen, Lebererkrankungen und Traumen. Verlaufskontrollen bei einigen Patienten weisen auch auf intraindividuelle Variation der spezifischen inhibitorischen Aktivität hin.

Introduction

Nine proteinase inhibitors, mainly glycoproteins in the relative molecular mass range between 54000 (α_1-proteinase inhibitor) and 725000 (α_2-macroglobulin), have so far been purified from human plasma and characterized by physico-chemical criteria (1—4). They play an important role in controlling the action of proteinases in fluids and tissues and guarantee homeostasis of enzyme systems which involve the specific and limited action of certain proteinases, e.g. in coagulation and fibrinolysis, complement cascade and proenzyme activation, and inflammatory processes with connective tissue destruction by leukocyte elastase and collagenase (5). The two major proteinase inhibitors in human serum are α_1-proteinase inhibitor (α_1-antitrypsin) and α_2-macroglobulin. α_1-Proteinase inhibitor consists of a single, glycosylated polypeptide chain and inactivates only serine proteinases by forming a reversible equimolar enzyme-inhibitor complex which results in a complete inactivation of the proteinase (e.g. trypsin, chymotrypsin, elastase, collagenase, plasmin) (1, 2). α_2-Macroglobulin is a high molecular weight proteinase inhibitor protein which entraps the proteinase irreversibly by proteinase-induced change of its conformation (6—9). Its broad inhibitor specificity is directed towards nearly all endoproteinases. In the complex with α_2-macroglobulin the proteinase retains activity against low molecular weight substrates (10). The pathogenetic and diagnostic significance of the two proteinase inhibitors necessitates simple, fast, reliable and sensitive assay procedures. The two proteins are determined routinely with immunologic techniques like radial immuno-diffusion, rocket immuno-electrophoresis, turbidimetry, and nephelometry (11). More recently, chromogenic substrates have been introduced which offer the possibility of determining the functional, i.e. the proteinase inhibitor activity, of the two proteins (12—15). Although the amidolytic assay with the chromogenic substrates has been evaluated and compared with immunological methods in sera of healthy persons (13—15), pathologic specimens have not been studied extensively so far. This, however, is important for routine clinical-chemical analysis and might give some hints on the pathophysiological role of proteinase inhibitor in serum. Therefore we compared both methods in a large number of pathological sera and calculated the specific proteinase inhibitor activities, i.e. the number of inhibitor units per g inhibitor protein, under normal and pathological conditions. The results point to significant changes of the specific inhibitor activity in certain diseases and intraindividually during the course of a disease.

Materials and Methods

Materials

All reagents (LN-antisera, control and standard sera) for laser immunonephelometric determinations were purchased from Behring Werke AG, Marburg; PreciChrom I/II was from Boehringer, Mannheim.

Specimens

Sera were obtained from the routine clinical-chemical analysis program of the central laboratory and classified according to the following disease categories: carcinoma (mainly breast cancer and bronchial carcinoma), diabetes mellitus, myocardial infarction, liver diseases (acute and chronic hepatitis, cholestasis, liver cirrhosis), trauma, and renal diseases (nephrotic syndrome, glomerulonephritis, renal insufficiency). The number of patients studied is listed in table 3 and 4. Biochemically and clinically healthy blood donors (n = 50), mainly male individuals ranging from 18 to 35 years, served as reference population. The specimens were stored for a maximum of 3 weeks at −20 °C before use.

Immunological determination of α_1-proteinase inhibitor and α_2-macroglobulin

Both proteins were determined laser nephelometrically by use of a fully automated He-Ne laser nephelometer (Behring Werke AG) (16, 17). The assays were perfomed according to the instructions of the manufacturer.

Functional determination of α_1-proteinase inhibitor and α_2-macroglobulin

Both proteins were determined by a chromogenic substrate assay (Boehringer Mannheim), the principles of which have been described in detail previously (12–15, 18).

α_1-Proteinase inhibitor was assayed in 1:11 diluted serum with N-benzoyl-*D*,*L*-arginine-*p*-nitroanilide (BAPA) as chromogenic substrate. The method was adapted to the analyser ACP 5040 Eppendorf but otherwise performed according to the instructions of the manufacturer.

α_2-Macroglobulin was determined in 1:21 diluted serum with carbobenzoxy-valyl-glycyl-arginine-*p*-nitroanilide (Chromozym TRY) as chromogenic substrate. The assay was performed manually with an Eppendorf Photometer 1101 M. The test volume was reduced by 50%, but otherwise the instructions of the manufacturer were followed.

The functional concentrations of the proteinase inhibitor are expressed as IU/l. One inhibitor unit (IU) inhibits 1 U of the respective proteinase, 1 U of the proteolytic enzyme turns over 1 µmol substrate per min at 25 °C.

The calculated specific proteinase inhibitor activity is defined as the number of inhibitor units (IU) per g inhibitor protein.

Determination of enzyme catalytic activities

The catalytic activities of alanine aminotransferase (EC 2.6.1.2) (19), aspartate aminotransferase (EC 2.6.1.1) (19), alkaline phosphatase (EC 3.1.3.1) (19) and γ-glutamyltranspeptidase (EC 2.3.2.2) (20) were determined according to optimized standard conditions adapted to an Eppendorf enzyme automate ACP 5040.

Statistical analyses

The significance of differences was tested with the *Mann-Whitney-Wilcoxon* procedure. The distribution mode of normal values was checked with the *Kolmogorov-Smirnov* test (21).

Results

Analytic criteria

Whereas the antigenic properties of α_1-proteinase inhibitor and α_2-macroglobulin are relatively stable, the inhibitory activities of these two proteins (as measured by the amidolytic substrate assay) are markedly influenced by the storage conditions of the sera. The functional activity of α_2-macroglobulin and to a lesser extent of α_1-proteinase inhibitor declines strongly and rapidly during storage at room temperature (+20 °C). This effect was less marked at lower storage temperatures (fig. 1). During 10 days at +4 °C and about 3 weeks at −20 °C full recovery of α_2-macroglobulin is guaranteed. α_1-Proteinase inhibitor always proved to be more stable than α_2-macroglobulin. It is noteworthy that after 3 weeks at −20 °C the functional activity of α_2-macroglobulin begins to decline continuously whereas that of α_1-proteinase inhibitor remains constant for at least 20 weeks. The within-run and day-to-day precision of both the amidolytic and immunologic determinations of the proteinase inhibitors are summarized in table 1. The precision of the functional assay of α_2-macroglobulin was lower than that of α_1-proteinase inhibitor, which might be due to the manual proce-

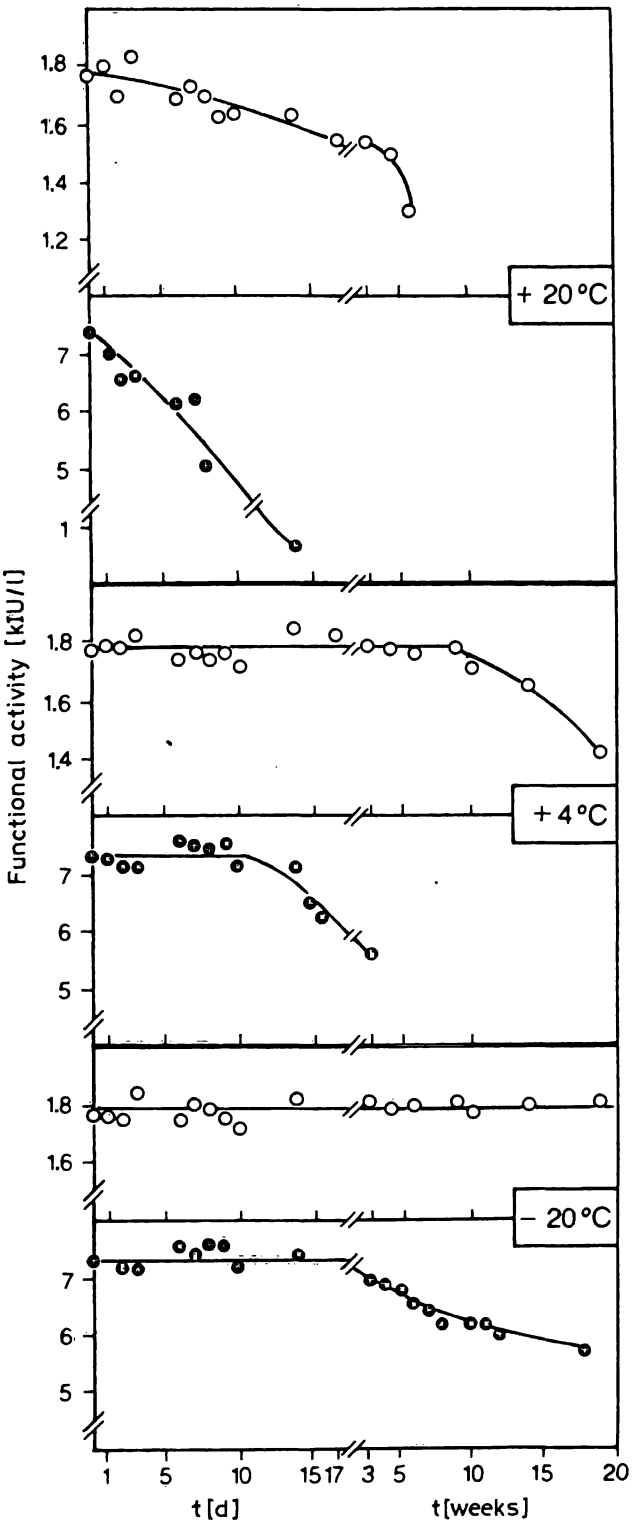

Fig. 1. Effect of storage time and temperature (+20 °C, +4 °C, −20 °C) on the functional activity of human serum α_1-proteinase inhibitor (O—O) and α_2-macroglobulin (●—●), determined with the chromogenic substrate assay.

dure of the former and the mechanization of the latter. With laser nephelometry the determination of α_1-proteinase inhibitor proved to be more precise than that of α_2-macroglobulin, whereas no significant difference in the precision of both assay principles was found.

The accuracies of both the functional and immunological assays are similar. The inaccuracies were 1 to 2.8% with only minor differences between α_1-proteinase inhibitor and α_2-macroglobulin.

In sera of normal persons the coefficients of correlation between the amidolytic and functional assay of α_1-proteinase inhibitor and α_2-macroglobulin were r = 0.988 and r = 0.916, respectively (fig. 2a, 3a). It should be noted, and will be shown in more detail below, that the correlations are worse for patients than for healthy persons (fig. 2b, 3b).

Tab. 1. Within-run (n = 20) and day-to-day (n = 20) imprecision of the functional and immunological determination of α_1-proteinase inhibitor and α_2-macroglobulin. Precichrom I was used for functional determinations the human control plasma, and human serum for the immunological determination. α_1-Proteinase inhibitor was assayed with the analyser ACP 5040 (Eppendorf); α_2-macroglobulin was determined manually with an Eppendorf Photometer. Immunological determinations were performed with a laser nephelometer.

	α_1-Proteinase inhibitor			α_2-Macroglobulin		
Assay procedure	$\bar{x}$	s	CV	$\bar{x}$	s	CV
Functional	(kIU/l)	(kIU/l)	(%)	(kIU/l)	(kIU/l)	(%)
within-run	1.42	0.03	2.07	4.43	0.20	4.6
day-to-day	1.32	0.06	4.24	4.68	0.32	6.8
Immunologic	(g/l)	(g/l)	(%)	(g/l)	(g/l)	(%)
within-day	2.48	0.07	2.70	1.73	0.08	4.8
day-to-day	2.30	0.11	4.86	1.76	0.11	6.3

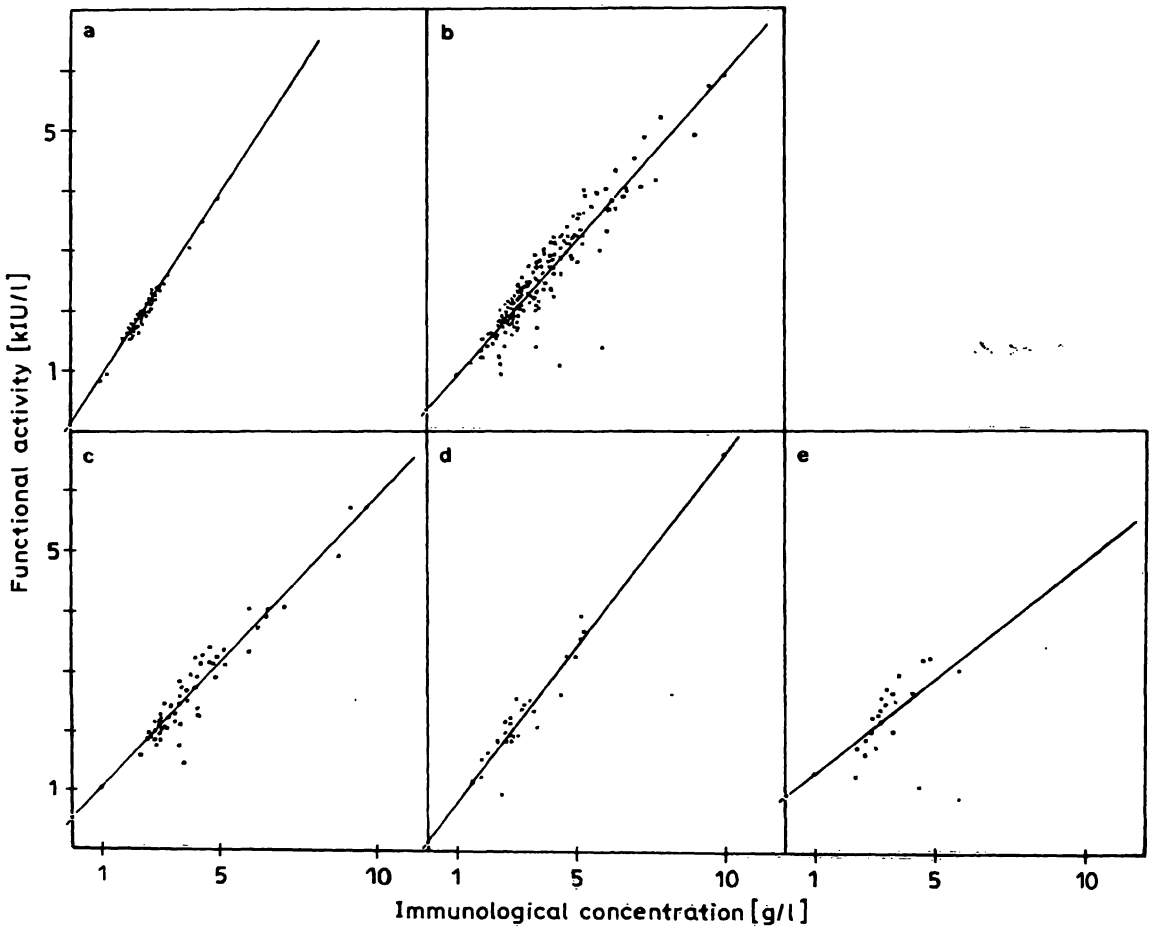

Fig. 2. Statistical correlation between the functional activity (chromogenic substrate assay) and immunological concentration (laser nephelometry) of α_1-proteinase inhibitor in human serum from
(a) normal persons (n = 50, r = 0.988, y = 0.760x + 0.054),
(b) all patients (n = 183, r = 0.907, y = 0.559x + 0.394),
(c) patients with carcinoma (n = 61, r = 0.942, y = 0.525x + 0.507),
(d) renal diseases (n = 29, r = 0.933, y = 0.647x + 0.157), and
(e) liver diseases (n = 22, r = 0.586, y = 0.394x + 0.928).

Reference ranges of functional concentration and specific inhibitor activity of α_1-proteinase inhibitor and α_2-macroglobulin

The functional and immunological concentrations and the specific inhibitor activities (proteinase inhibitor activity/g protein) of α_1-proteinase inhibitor and α_2-macroglobulin in sera of normal persons (n = 50) exhibit a *Gauss*ian distribution (not shown). The reference ranges (± 2 S.D.) of the concentrations and of calculated specific inhibitor activities are listed in table 2.

Tab. 2. Reference ranges (± 2 S.D.) of functional (proteinase inhibitor) activity, immunological concentration, and specific inhibitor activity of α_1-proteinase inhibitor and α_2-macroglobulin in serum (n = 50).

Proteinase inhibitor	Functional activity (kIU/l)	Immunological concentration (g/l)	Specific inhibitor activity (kIU/g)
α_1-Proteinase inhibitor	1.17–2.78	1.48–3.58	0.73–0.84
α_2-Macroglobulin	3.59–9.14	1.03–2.36	3.12–4.53

Functional concentration and specific inhibitor activities of α_1-proteinase inhibitor and α_2-macroglobulin in patients with various diseases

Using sera from patients afflicted with carcinoma, diabetes mellitus, renal and liver diseases, myocardial infarction and trauma, the two proteinase inhibitors were measured with the amidolytic and immunologic method and the specific inhibitor activity was calculated. As summarized in table 3 the specific inhibitor activity of α_1-proteinase inhibitor showed a statistically significant ($p < 0.05$) decrease in all dis-

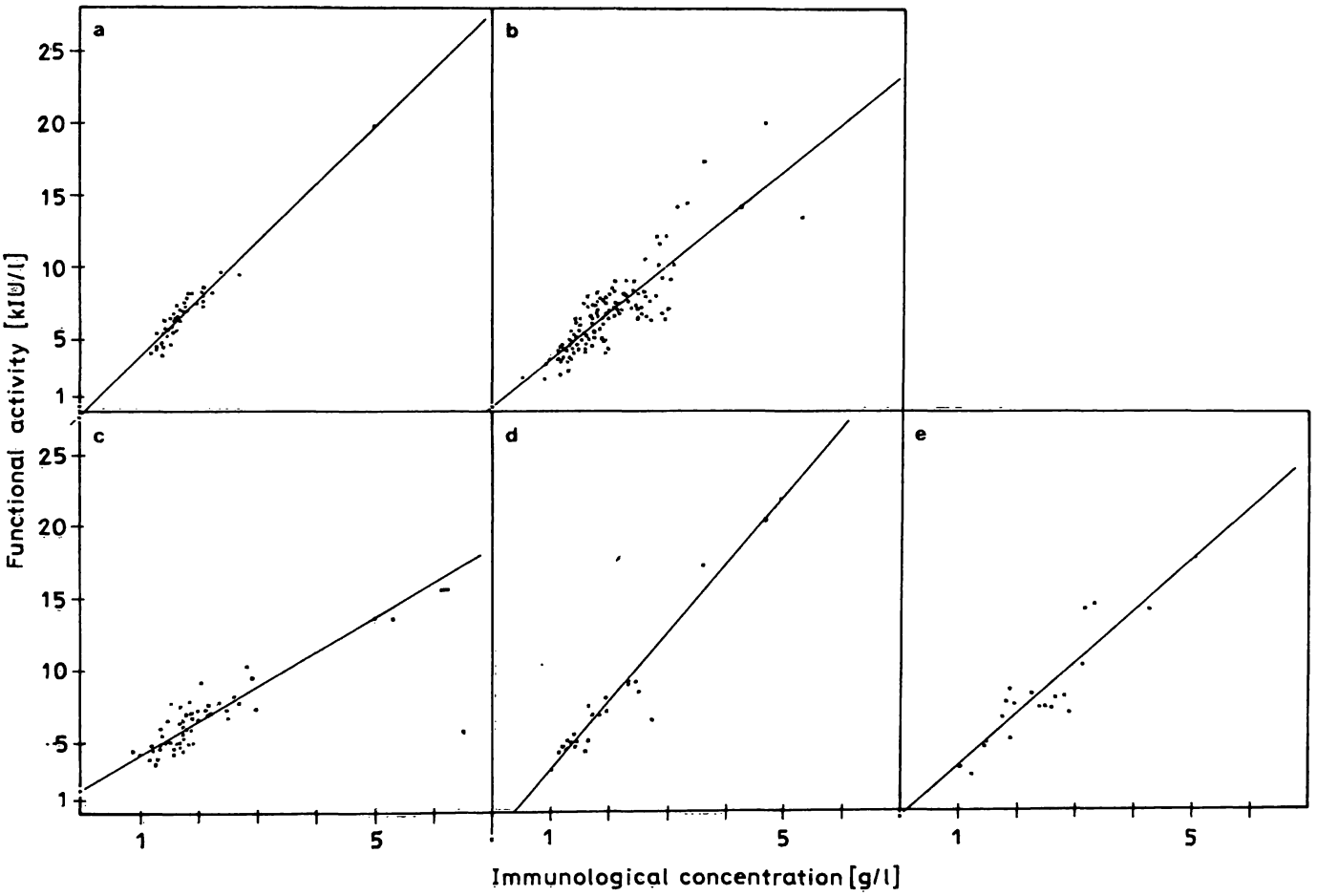

Fig. 3. Statistical correlation between the functional activity (chromogenic substrate assay) and immunological concentration (laser nephelometry) of α_2-macroglobulin in human serum from
(a) normal persons (n = 50, r = 0.916, y = 4.025x − 0.316),
(b) all patients (n = 153, r = 0.862, y = 3.36x + 0.259),
(c) patients with carcinoma (n = 49, r = 0.858, y = 2.411x + 1.694),
(d) renal diseases (n = 23, r = 0.978, y = 4.773x − 1.730), and
(e) liver diseases (n = 19, r = 0.852, y = 3.616x − 0.380).

Tab. 3. Mean values ± s.D. of functional activity (kU/l), immunological concentrations (g/l) and specific inhibitor activity (kIU/g) of α_1-proteinase inhibitor in healthy persons and patients with various diseases. The statistical significance of the differences between healthy persons and patients was tested (n.s. = not significant).

Disease category	Method	Number	Mean values	S.D.	Level of significance
Healthy persons	functional	50	1.97	0.40	
	immunologic	50	2.53	0.53	
	specific inhibitor act.	50	0.78	0.03	
All patients	functional	197	2.49	0.87	$p < 0.001$
	immunologic	183	3.80	1.39	< 0.001
	specific inhibitor act.	183	0.67	0.09	< 0.001
Carcinoma	functional	67	2.63	0.87	$p < 0.001$
	immunologic	61	4.07	1.49	< 0.001
	specific inhibitor act.	61	0.66	0.08	< 0.001
Diabetes mellitus	functional	18	2.11	0.62	n.s.
	immunologic	15	3.19	0.89	$p < 0.01$
	specific inhibitor act.	15	0.71	0.05	< 0.001
Renal disease	functional	30	2.22	0.73	n.s.
	immunologic	29	3.22	1.06	$p < 0.01$
	specific inhibitor act.	29	0.70	0.09	< 0.001
Liver disease	functional	22	2.32	0.59	$p < 0.01$
	immunologic	22	3.53	0.87	< 0.001
	specific inhibitor act.	22	0.67	0.13	< 0.001
Myocardial infarction	functional	23	2.42	0.95	n.s.
	immunologic	22	3.61	1.32	$p < 0.001$
	specific inhibitor act.	22	0.67	0.09	< 0.001
Trauma	functional	23	2.82	1.02	$p < 0.05$
	immunologic	22	4.29	1.58	< 0.01
	specific inhibitor act.	22	0.68	0.06	< 0.001

Tab. 4. Mean values ± S.D. of functional activity (kIU/l), immunological concentrations (g/l) and specific inhibitor activity (kIU/g) of α_2-macroglobulin in healthy persons and patients with various diseases. The statistical significance of the differences between healthy persons and patients was tested (n.s. = not significant).

Disease category	Method	Number	Mean values	S.D.	Level of significance
Healthy persons	functional	50	6.50	1.46	
	immunologic	50	1.69	0.33	
	specific inhibitor act.	50	3.83	0.35	
All patients	functional	195	6.79	2.64	n.s.
	immunologic	153	1.92	0.70	n.s.
	specific inhibitor act.	153	3.49	0.62	< 0.001
Carcinoma	functional	67	6.52	1.93	n.s.
	immunologic	49	1.91	0.69	n.s.
	specific inhibitor act.	49	3.36	0.57	$p < 0.001$
Diabetes mellitus	functional	18	7.01	2.18	n.s.
	immunologic	14	1.87	0.47	n.s.
	specific inhibitor act.	14	3.62	0.46	n.s.
Renal disease	functional	30	7.74	3.96	n.s.
	immunologic	23	1.90	0.83	n.s.
	specific inhibitor act.	23	3.78	0.49	n.s.
Liver disease	functional	22	8.19	3.23	$p < 0.05$
	immunologic	19	2.37	0.77	< 0.01
	specific inhibitor act.	19	3.44	0.72	< 0.05
Myocardial infarction	functional	23	5.59	1.85	$p < 0.05$
	immunologic	20	1.65	0.63	n.s.
	specific inhibitor act.	20	3.55	0.62	n.s.
Trauma	functional	23	5.36	1.75	$p < 0.01$
	immunologic	17	1.60	0.41	n.s.
	specific inhibitor act.	17	3.31	0.64	$p < 0.01$

ease conditions, whereas the mean concentration and activity were increased in all groups. It is obvious that the elevation of the functional activity is less pronounced than that of the immunological concentration. Extreme reductions of the specific inhibitor activity of α_1-proteinase inhibitor (0.24 kIU/g) were found in patients with acute pancreatitis and liver cirrhosis. However, this finding is not typical for these diseases, since other patients with similar lesions did not show such a marked reduction of the specific activity. No significant differences of the specific inhibitor activities were found between the various disease groups.

The statistic correlation of the functional and immunologic assay of α_1-proteinase inhibitor in diseased individuals is lower than that in healthy persons (fig. 2). A marked discrepancy in the results of both methods was observed in patients with acute and chronic liver diseases ($r = 0.586$). The specific inhibitor activities of α_2-macroglobulin and of α_1-proteinase inhibitor were decreased in most patients but statistically significant changes ($p < 0.05$) were found only in carcinoma, liver diseases, and trauma (tab. 4). Very large decreases of specific inhibitor activity were measured in sera of some patients with acute pancreatitis (2.22 kIU/g) and CCl_4 poisoning (2.23 kIU/g), whereas highly elevated specific inhibitor activities were found in patients with Morbus *Hodgkin* (4.98 kIU/g), sepsis (4.87 kIU/g), and nephrotic syndrome (4.81 kIU/g). However, it should be emphasized that these changes are neither disease-typical nor disease-obligatory laboratory findings.

The statistical correlation between both assay principles was lower in patients than in healthy persons. This effect is pronounced in individuals afflicted with liver diseases ($r = 0.852$) and carcinoma ($r = 0.858$) (fig. 3).

In some cases, concentration, inhibitor activity and specific inhibitor activity of both proteinase inhibitors were monitored during the course of a disease.

A patient with acute carbon tetrachloride poisoning, showing extremely elevated catalytic activities of both aminotransferases in serum, exhibited a rather constant specific activity of α_1-proteinase inhibitor in the normal range. The specific activity of α_2-macroglobulin, however, was initially significantly reduced (2.2 kIU/g) but increased to normal values when the aminotransferases declined (fig. 4). This change is due to a selective, strong increase of the inhibitor activity of α_2-macroglobulin, with only a slight increase in the concentration of the protein.

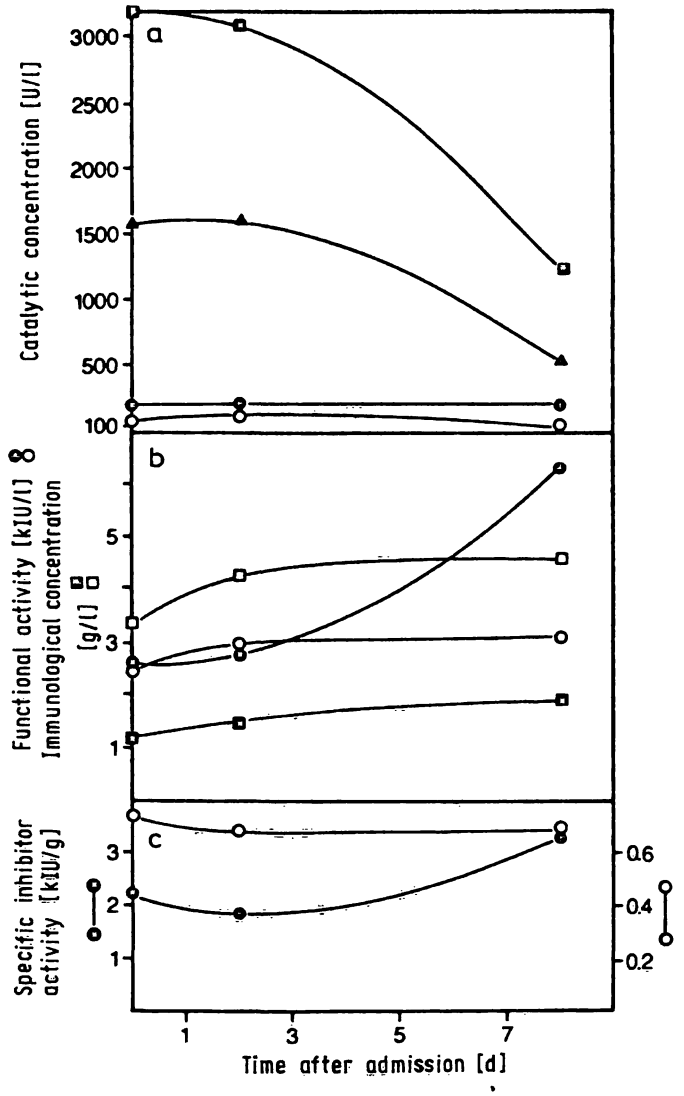

Fig. 4. Follow up of the functional (inhibitor) activities and immunological concentrations of α_1-proteinase inhibitor and α_2-macroglobulin in serum of a patient with acute CCl_4 poisoning.
(a) catalytic concentrations of alanine-aminotransferase (■—■), aspartate-aminotransferase (▲—▲), alkaline phosphatase (●—●) and γ-glutamyltranspeptidase (○—○) are shown.
(b) functional activity (kIU/l) (●, ○) and immunological concentration (g/l) (■, □), of α_1-proteinase inhibitor (○, □) and α_2-macroglobulin (●, ■).
(c) calculated specific inhibitor activity of α_1-proteinase inhibitor (○—○) and α_2-macroglobulin (●—●).

Similar, but uncharacteristic changes of the specific inhibitor activity of α_2-macroglobulin were also noticed in the postoperative course and in patients with hepatocellular carcinoma (not shown). These longitudinal studies show that the immunoreactive concentrations and functional activities of the proteinase inhibitors do not behave strictly in parallel during the course of a disease in an individual.

Discussion

The introduction of chromogenic substrates provides an alternative, i.e. functional approach to the determination of α_1-proteinase inhibitor and α_2-macroglobulin which is suitable for routine clinical chemical analyses (12—15). The analytic criteria of the amidolytic assay are comparable to those of methods measuring the antigenic levels of the proteinase inhibitors. In our study the precision of α_2-macroglobulin determination was lower than that of α_1-proteinase inhibitor, irrespective of the assay principle; but other authors have reported precision data which are similar to those found for α_1-proteinase inhibitor (15). The storage conditions of the sera must be controlled carefully if both proteins are determined amidolytically. α_2-Macroglobulin is obviously more susceptible to in vitro inactivation than α_1-proteinase inhibitor. By use of the amidolytic assay additional information on the functional and pathogenetic significance of the two proteinase inhibitors can be obtained. Whereas immunologic procedures are unable to distinguish between active, non-active and inactivated protein molecules the chromogenic substrate assay measures specifically the biologically relevant, i.e. the antiproteolytic active fraction of the protein. In agreement with previous reports, healthy persons showed a satisfactory statistical correlation between the immunoreactive and functional concentrations of the proteins (13—15), and the reference range for the functional concentration of α_2-macroglobulin was similar to that reported previously (15). In patients, however, the correlations are lower, especially in those suffering from acute and chronic liver diseases (α_1-proteinase inhibitor and α_2-macroglobulin) and carcinoma (α_2-macroglobulin). The reason for this discrepancy is not known at present but variable degrees of endogenous complex formation between the inhibitor protein and certain proteinases in serum might be relevant.

Data summarized in table 3 and 4 support the view that α_2-macroglobulin, in contrast to α_1-proteinase inhibitor, does not behave like an acute phase reactant in humans (22). The specific inhibitor activity of the macroglobulin is changed only insignificantly un-

der various disease conditions, whereas that of α_1-proteinase inhibitor is significantly reduced in all disease categories. This is due to differences between the relative increase of the functional activity and immunoreactive concentration in acute phase reactions (e.g. trauma, carcinoma, myocardial infarction). The finding might be explained by two mechanisms.

a) accumulation of functional inactive fraction of α_1-proteinase inhibitor, which retains full immunoreactivity under these conditions. The inactivation of α_1-proteinase inhibitor might be produced by oxidation of a methionine residue near the proteinase inhibitory site, possibly by oxygen free radicals (23–25). It is known that oxygen free radicals generated by complement activated neutrophils or other processes are important mediators of tissue injury in a great variety of diseases (26, 27).

b) Variable degrees of endogenous saturation of the proteinase inhibitor capacity. α_1-Proteinase inhibitor constitutes 90% of human serum elastase inhibitor capacity (4). This proteinase (together with others) is released from neutrophils during inflammation and is consequently trapped by α_1-proteinase inhibitor, leaving it antigenically intact but functionally inactive.

It is not possible to decide which of the two mechanisms is operative under various disease conditions. The relative constancy of the specific inhibitor activity of α_2-macroglobulin can be explained by the effective hepatic clearance of even low concentrations of α_2-macroglobulin-proteinase complexes from the circulation with a half-life in the order of 3 to 4 minutes (28, 29), whereas that of α_2-macroglobulin itself is much longer (30). This mechanism prevents the accumulation of proteinase-burdened, i.e. inactive α_2-macroglobulin in the circulation.

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